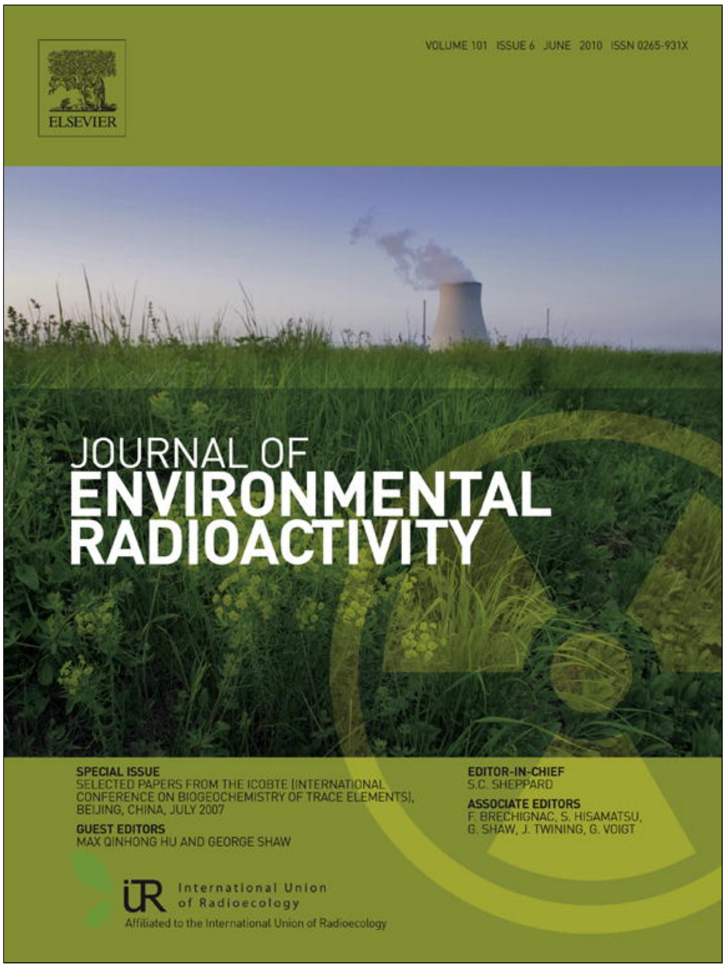
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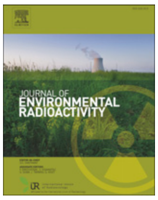

Biosorption of uranium (VI) by immobilized *Aspergillus fumigatus* beads

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ABSTRACT

Biosorption of uranium (VI) ions by immobilized *Aspergillus fumigatus* beads was investigated in a batch system. The influences of solution pH, biosorbent dose, U (VI) concentration, and contact time on U (VI) biosorption were studied. The results indicated that the adsorption capacity was strongly affected by the solution pH, the biosorbent dose and initial U (VI) concentration. Optimum biosorption was observed at pH 5.0, biosrobent dose (w/v) 2.5%, initial U (VI) concentration 60 mg L^{-1}. Biosorption equilibrium was established in 120 min. The adsorption process conformed to the Freunlich and Temkin isothermal adsorption models. The dynamic adsorption model conformed to pseudo-second order model.

1. Introduction

Every year, large amounts of wastewater containing uranium (U) is produced by the nuclear industry, ore mining and industries that apply radioactive isotopes. Uranium disposed into the environment can eventually reach the top of the food chain and be ingested by humans, causing severe kidney or liver damage and even death (Xie et al., 2008). Moreover, from the point of resource recovery, the removal and recovery of metals is important as metals are non-renewable (Puranik et al., 1999).

Conventional treatments of metal effluents are either ineffective, costly, complicated or have sludge disposal problems. Biosorption is regarded as an innovative technology to remove metals from aqueous solution because it has some obvious advantages (Chen and Gan, 1999; Kratochvil and Volesky, 1998a,b): high efficiency and selectivity for absorbing metals in low concentrations, energy-saving, broad operational range of pH and temperature, easy reclamation of metals, and easy recycling of the biosorbent.

Fungal biosorption has been studied extensively because of the availability of large amounts of waste fungal biomass from fermentation industries and the amenability of the microorganisms to genetic and morphological manipulation (Zhou and Kiff, 1991). Important fungal biosorbents include *Aspergillus*, *Rhizopus* and *Penicillium*. A recent study demonstrated that *Aspergillus fumigatus* could be used as biomass for biosorption of U ions (Bhainsa and D'Souza, 1999).

The use of freely suspended biomass may be plagued with operational difficulties, but immobilized microbial cell systems could provide additional advantages: ease of regeneration and reuse of the biomass, easier solid–liquid separation and minimal clogging in continuous flow systems (Bayramoğlu et al., 2003; Özdemir et al., 2005).

In this study, we used immobilized *A. fumigatus* beads as a biosorbent for U(VI) ions. The effect of solution pH, biosorbent dose, initial U(VI) concentration, and contact time on U(VI) removal was investigated in a batch system. The experimental results would provide worthy information on application of this adsorbent in treatment of U-contained wastewater.

2. Materials and methods

2.1. Main instruments and materials

A PHS-3C precision pH meter, a GB303 electronic balance, a HD2-HZQ vertical temperature oscillator, a YXQ-LS-18SI automatic portable sterilizer and a 10 mL injector were used in the experiments. The concentration of the residual U (VI) in the solution was determined with microtitration as described in our previous study (Xie et al., 2009). *A. fumigatus* was obtained from Guangdong Institute of Microbiology, China. Culture medium used in this study was (g/L) glucose 15, KH_2PO_4 1, $(NH)_2SO_4$ 1, $MgSO_4 \cdot 7H_2O$ 0.5, NaCl 0.5, and yeast extract 1. The immobilization carrier, sodium

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alginate (SA), was provided by Tianjin Guangfu Fine Chemical Research Institute, China.

2.2. Preparation of spore suspension

The strain was inoculated on a culture medium slant for 5 days and transferred into conical flasks containing culture and glass beads, and surged on a rotary shaker (180 r/min) at 30 °C for 5 h, diluted to 10^7/ml (Wang and Hu, 2007).

2.3. Immobilization of *Aspergillus fumigatus*

As described in the literature (Wang et al., 2008), 100 mL of SA solution (3.0%) and 1 mL of spore suspension was mixed. The mixture was injected drop-wise into a $CaCl_2$ solution (4%) using an injector to form beads. The spore-immobilized beads were cured in the $CaCl_2$ solution for 4 h to enhance their mechanical stability. The beads were rinsed and transferred into culture medium in conical flasks. After 3 days of shaking on a rotary shaker (180 r/min) at 30 °C, the active immobilized *A. fumigatus* beads were collected and rinsed as biosorbent for use. Blank beads without *A. fumigatus* served as control. The photos of the active immobilized *A. fumigatus* beads and blank beads are shown in Fig. 1.

2.4. Biosorption procedures

For all the biosorption experiments, the active SA immobilized *A. fumigatus* beads were introduced into 50 mL of the U (VI) solution in conical flasks, and the initial pH was adjusted. Then the experiments continued on a rotary shaker (180 r/min) at 30 °C. The concentration of the residual U (VI) ions in the solution was measured as described above. Each of the experiment was repeated thrice and the average values were obtained.

The percentage removal and the amount of adsorbed U (VI) onto the unit amount of the biosorbent (mg U per g dry biosorbent) were obtained by using the following expressions:

$$\text{Percentage removal}(\%) = [(C_0 - C)/C_0] \times 100\% \tag{1}$$

$$q = [(C_0 - C)V]/M \tag{2}$$

Where q (mg/g) is the amount of U (VI) adsorbed onto the unit amount of the adsorbent, C_0 and C (mg/L) are the concentrations of the U (VI) in the solution before and after biosorption, respectively. V (L) is the volume of the aqueous solution and M (g) is the dry weight of the biosorbent. In all the experiments, the dry/wet weight ratio of the immobilized fugal beads was measured to be 3.5 ± 0.3%.

2.5. Equilibrium isotherm and kinetics studies

Langmuir, Freundlich and Temkin models were used to determine the sorption equilibrium between the biosorbent and metals ions. The isotherm equations for all the models are listed in Table 1 (Mashitah et al., 2008). The isotherm constants for the three models can be obtained by non-linear regression methods (Basha et al., 2008; Isik, 2008).

Table 1
Equilibrium isotherm models.

Models	Equation	Linear equation	References
Langmuir	$q = q_{\max}K_{\mathrm{L}}c/(1 + K_{\mathrm{L}}c)$	$\frac{1}{q} = \frac{1}{q_{\max}} + \frac{1}{K_{\mathrm{L}}q_{\max}} \times \frac{1}{c}$	Aksu (2002); Malkoc and Nuhoglu (2005)
Freundlich	$q = K_{\mathrm{F}}c^{\frac{1}{n}}$	$\ln q = \frac{1}{n}\ln c + \ln K_{\mathrm{f}}$	Arica et al. (2001)
Temkin	$q = \frac{RT}{b_{\mathrm{T}}}(\ln a_{\mathrm{T}}c)$	$q = \frac{RT}{b_{\mathrm{T}}}\ln a_{\mathrm{T}} + \frac{RT}{b_{\mathrm{T}}}\ln c$	Basha et al. (2008); Isik (2008)

Different types of kinetic models including pseudo-first order, pseudo-second order and intraparticle diffusion were used to investigate the mechanism of biosorption and potential rate controlling steps such as mass transport and chemical reaction processes (Mashitah et al., 2008). The pseudo-first order equation is given as:

$$\log(q_e - q_t) = \log q_e - \frac{k_1}{2.303}t \tag{3}$$

Where q_e and q_t (mg/g) are the amounts of adsorbed uranyl ions on the biosorbent at equilibrium and at time t, respectively, and k_1 (min^{-1}) is the pseudo-first order biosorption rate constant.

The pseudo-second order equation is given as:

$$\frac{t}{q_t} = \frac{1}{k_2 q_e^2} + \frac{1}{q_e}t \tag{4}$$

Where k_2 (g/mg min) is the second-order biosorption rate constant, and q_e (mg/g) is the biosorption capacity calculated by the pseudo-second order kinetic model.

3. Results and discussion

3.1. Effect of solution pH

Solution pH plays an important role in the biosorption of U from the aqueous solutions. It influences both the speciation of U in the aqueous solution and the binding sites present on the surface of biomass (Uslu and Tanyol, 2006). The effect of pH on U biosorption onto the immobilized *A. fumigatus* beads was studied in order to find out the optimum pH for the biosorption process, and to find out whether the biomass was able to show a good U (VI) uptake at extreme pH values (Bhat et al., 2008).

The percentage removal and adsorption capacity of U versus pH is plotted in Fig. 2, which resulted in a bell shaped curve. As seen from the figure, the maximum percentage removal of U was obtained at pH 5.0 and the minimum percentage removal of U was observed at pH 2.0. When the solution pH is 2.0, there is a high concentration of H^+ and H_3O^+, which compete with other ions (uranyl) for the binding sites on the surface of the biomass (Sar and D'Souza, 2002), resulting in a decreased biosorption of U

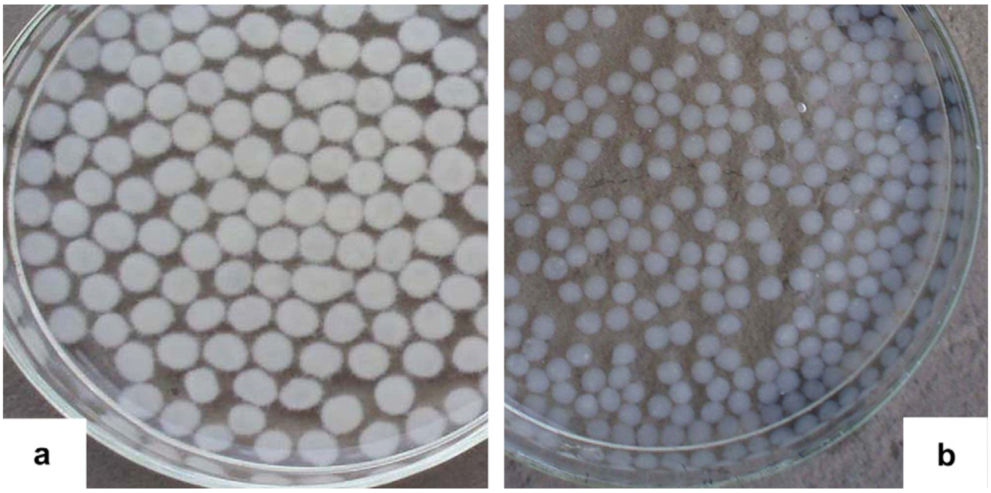

Fig. 1. (a) Immobilized *Aspergillus fumigatus* beads. (b) Blank beads.

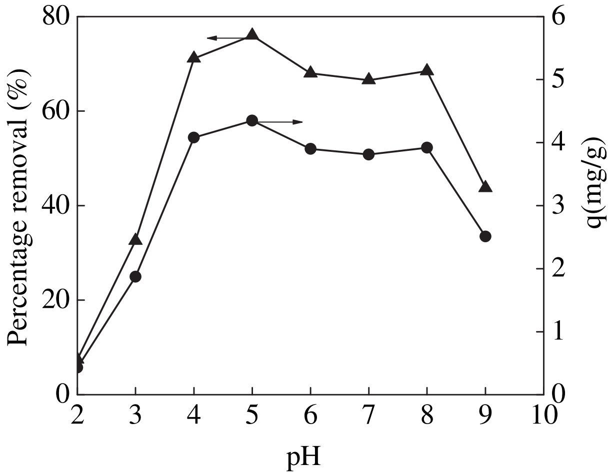

Fig. 2. Biosorption of U at different pH. Conditions: initial U (VI) concentration, 10 mg L^{-1}; contact time, 60 min; temperature, 30 °C; dose of wet beads, 2.0% (w/v).

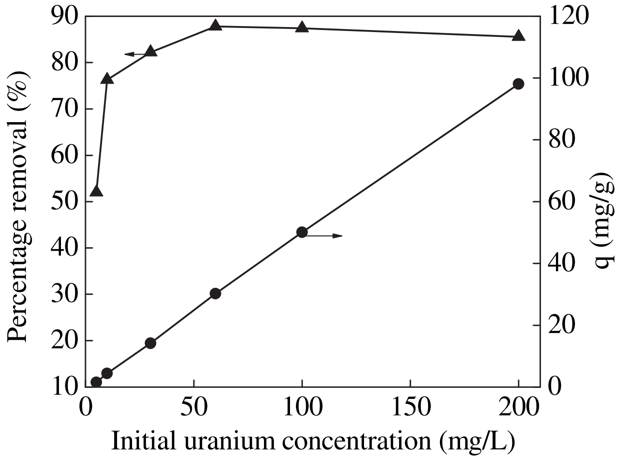

Fig. 4. Biosorption of U at different initial U concentrations. Conditions: initial pH, 5.0; dose of wet beads, 2.0% (w/v); contact time, 60 min; temperature, 30 °C.

at this pH value. Optimum biosorption at pH 5.0 could be due to the presence of ligands such as carboxyl, amino, and phosphate on the surface of beads. Decrease in the uptake of U at higher pH could be due to the formation of uranyl complexes, such as UO_2OH^+, $(UO_2)_2(OH)_2^{2+}$ and $(UO_2)_3(OH)_5^+$. Moreover, because of the endogenous respiring of the immobilized biomass, there may carbonate produced. Carbonate and atmospheric CO_2 result in the formation of uranyl carbonate complexes when pH is over 6.0 (Krestou and Panias, 2004). The uranyl carbonate complexes compete with surface binding sites for uranyl ions and reduce the availability of U for biosorption (Wazne et al., 2006). Also at higher pH formation of solid schoepite ($4UO_3 \cdot 9H_2O$) takes place which decreases the dissolved U concentration in solution, and consequently leads to the reduced sorption of U onto the biomass (Saxena et al., 2006) The optimum pH was found to be 5, and all the subsequent experiments were conducted at this pH value.

3.2. Effect of biosorbent dose

The experiments were carried out by varying the biosorbent loading from 1.0% (w/v) to 3.5%. The percentage removal and adsorption capacity of U (VI) at different biosorbent concentrations are presented in Fig. 3. As shown in the figure, the percentage removal increased with the increase of biosorbent dose, this is due to the increased surface area of the biosorbent, which in turn increases the number of binding sites (Esposito et al., 2001). But the U ions uptake decreased from 7.2 to 2.7 mg/g when the biosorbent loading increased from 1.0% to 3.5%. The higher biosorbent loading could produce a 'screen' effect on the cell wall, protecting the binding sites, thus resulting in lower U ions adsorption per unit of sorbent (Mashitah et al., 1999; Malkoc and Nuhoglu, 2005).

3.3. Effect of initial U concentration

The percentage removal and adsorption capacity at different U (VI) concentrations are presented in Fig. 4. When the initial metal ions concentration varied from 5 mg/L to 200 mg/L, the percentage of ions adsorbed at higher concentration levels showed a decreasing trend, whereas uptake of ions displayed the opposite trend. The maximum percentage removal of U (87.8%) was observed when the initial U concentration was 60 mg/L. The maximum U uptake capacity of this biosorbent was achieved at 98.4 mg U per g dry biosorbent with the initial U concentration of 200 mg/L. This may be due to the saturation of the adsorption sites and increase in the number of ions competing for the available binding sites in the biomass for complexation of U at higher

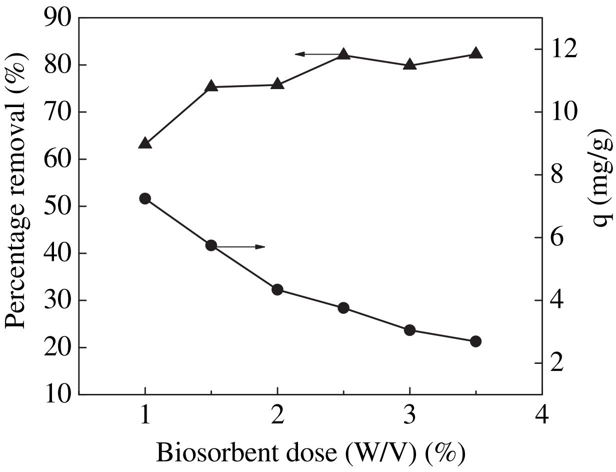

Fig. 3. Biosorption of U with different wet beads dose. Conditions: initial U concentration, 10 mg L^{-1}; initial pH, 5.0; contact time, 60 min; temperature, 30 °C.

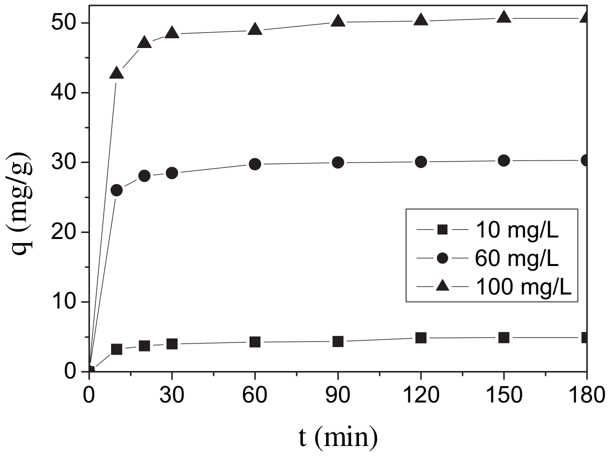

Fig. 5. Biosorption of U with contact time. Conditions: initial U (VI) concentration 10 mg L^{-1}; initial pH, 5.0; dose of wet beads, 2.0% (w/v); temperature, 30 °C.

Table 2
Percentage removal of U by blank beads and immobilized *Aspergillus fumigatus* beads.

Initial U concentration (mg/L)	Percentage removal of U (%)	
	Blank beads	Immobilized *Aspergillus fumigatus* beads
10	21.2 ± 1.9	77.4 ± 2.6
60	22.5 ± 2.1	87.8 ± 1.5
100	20.7 ± 1.7	85.8 ± 2.0

concentration (Bai and Abraham, 2001a, 2003). On the other hand, U (VI) adsorption increased with the increase of initial U (VI) concentration as higher concentration of metal ions enhanced the mass transfer driving force, and increased the metal ions adsorbed per unit weight of adsorbent at equilibrium (Aksu, 2002). In addition, increasing initial U concentration increased the number of collisions between U ions and the adsorbent, which enhanced the adsorption process (Bai and Abraham, 2001a,b; Aksu, 2002).

3.4. Effect of contact time

The biosorption rate of U (VI) is exemplified in Fig. 5. The results showed that the percentage removal of U (VI) by immobilized *A. fumigatus* beads increased from 56.3% to 85.5% when the contact time varied from 10 to 180 min. The biosorption process was very fast within 30 min and with 95% U uptake taking place within 60 min. Biosorption equilibrium was established within 120 min. It was also observed that the adsorption slowed down in later stages because initially a large number of vacant surface sites may be available for adsorption and after some time, the remaining vacant surface sites may be difficult to occupy due to repulsive forces between the solute molecules of the solid and bulk phase (Viswanadham et al., 2000; Saravanane et al., 2002).

3.5. Compare the uptake of U by immobilized *A. fumigatus* beads with blank beads

Under the experimental conditions with temperature 30 °C and solution pH 5.0, 2.0% (w/v) of wet beads were used to adsorb U with contact time of 60 min, the percentage removal of U with different initial U concentration was presented in Table 2. The percentage removal of U was 21.2%, 22.5% and 20.7% at 10, 60, 100 mg/L initial U concentrations after 1 h of adsorption by blank beads and it increased to 77.4%, 87.8% and 85.8% by immobilized *A. fumigatus* beads. The result indicated that the immobilized *A. fumigatus* beads performed better than the blank beads (Ca-alginate) for U adsorption, and Ca-alginate also added to U percentage removal. The Ca-alginate acted as both an adsorbent and a support material for the entrapment of *A. fumigatus*. The Ca-alginate has also been reported to enhance Cd uptake when it was used to entrap *Trametes versicolor* (Arica et al., 2001) and to enhance U uptake when it was used to immobilize *Trichoderma harzianum* (Akhtar et al., 2009) as compared with blank Ca-alginate beads.

Table 3
Biosorption equilibrium constants obtained from Langmuir, Freundlich and Temkin isotherms in the biosorption of U onto immobilized *Aspergillus fumigatus*.

Models	Fitting equation	Corresponding parameters
Langmiur	$y = 0.5897x - 0.0288$	$R^2 = 0.9725$, $q_{\max} = -34.7222$ mg/g, $K_L = -0.0488$
Freundlich	$y = 1.2566x + 0.5856$	$R^2 = 0.9625$, $n = 0.7958$, $K_f = 0.5568$
Temkin	$y = 36.403x - 35.526$	$R^2 = 0.9346$, $b_T = 69.2598$, $a_T = 0.3768$

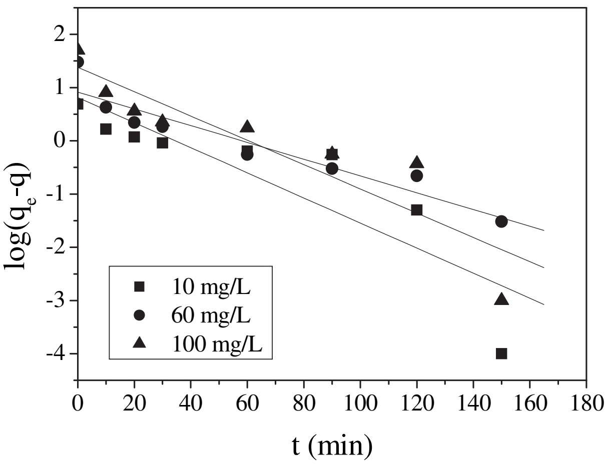

Fig. 6. Pseudo-first order sorption kinetic of U (VI) onto immobilized *Aspergillus fumigatus* beads at different initial U (VI) concentrations.

3.6. Adsorption isotherm

The constants of Langmuir, Freundlich and Temkin isotherms models for the U (VI) biosorption onto immobilized cells of *A. fumigatus* are presented in Table 3. Based on the correlation coefficient R^2, the Langmuir isotherm model was the best model to describe the experimental data, but the negative value of $q_{\max}$ indicated that this model is not suitable to be used in this case. Compared with the other two models, it is clear that both the Freundlich and Temkin isotherm models fitted with the experimental data well, and the former model was a better fit than the latter.

3.7. Biosorption kinetics

The linear form of the pseudo-first order model (Eq. (3)) and the pseudo-second order model (Eq. (4)) for the adsorption of U (VI) at various initial U (VI) concentrations is given in Figs. 6 and 7, respectively. The correlation coefficients (R^2) for the linear plots using the pseudo-first order model ranged between 0.764 and 0.899 while the correlation coefficients for the linear plots from the pseudo-second order model ranged between 0.996 and 0.999. This result suggested that the pseudo-first order model is less suitable to describe this biosorption process and the pseudo-second order

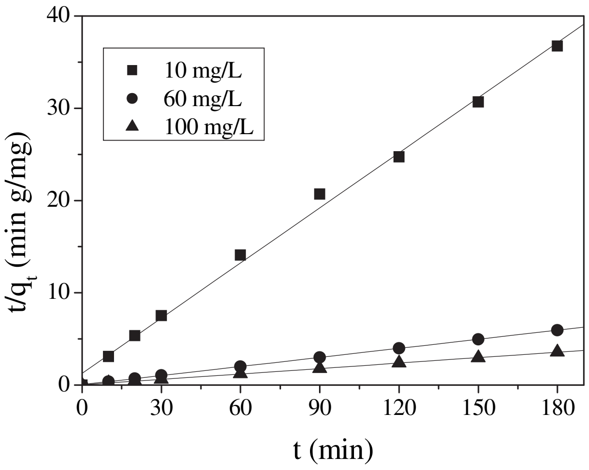

Fig. 7. Pseudo-second order sorption kinetic of U (VI) onto immobilized *Aspergillus fumigatus* beads at different initial U (VI) concentrations.

Table 4
Kinetic constants of pseudo-second order model and correlation coefficients (R^2) at 30 °C.

C_0 (mg/L)	Fitting equation	Corresponding parameters		
		K_2 (min g/mg)	q_e (mg/g)	R^2
10	$y = 0.1992x + 1.2677$	0.0313	5.02	0.996
60	$y = 0.0328x + 0.0457$	0.0235	30.49	0.999
100	$y = 0.0196x + 0.0293$	0.0131	51.02	0.999

model can be adapted to describe this process as it fit accurately with the experimental data. The kinetic constants of this model are presented in Table 4.

4. Conclusions

The results of present studies showed that U (VI) ions could be effectively adsorbed by the active immobilized *A. fumigatus* beads. Optimal biosorption was observed at initial pH 5.0. The adsorption capacity of U (VI) decreased with the increase of biosorbent dose. The percentage removal of U (VI) ions increased with the increase of initial U (VI) concentration in the range of 5–60 mg/L, and it decreased with increase of initial U (VI) concentration in the range of 60–200 mg/L. The maximum percentage removal of U (87.8%) was observed when the initial U (VI) concentration was 60 mg/L. The adsorption equilibrium was established after 120 min.

The Freundlich and Temkin adsorption models could be used to describe this adsorption process, suggesting that this process is a complicated multi-step form. The kinetic study showed that the pseudo-second order model is appropriate to describe the adsorption process, suggesting that this process could be a chemisorption process.

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